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(54) Title: MOLECULARLY IMPRINTED BEADED POLYMERS AND STABILIZED SUSPENSION POLYMERIZATION OF THE SAME IN PERFLUOROCARBON LIQUIDS (57) Abstract A suspension polymerization technique is provided for molecular imprinting based on the use of a perfluorocarbon liquid as the dispersing phase that does not interfere with the interactions between functional monomers and print molecules required for the recognition process during molecular imprinting, and provides controllable particle sizes within less than about 5 μ m to about 100 μ m.		

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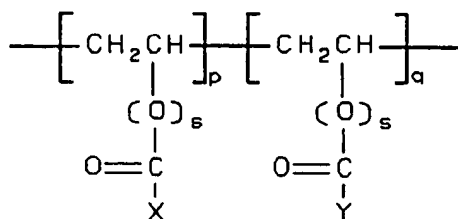
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**MOLECULARLY IMPRINTED BEADED POLYMERS AND
STABILIZED SUSPENSION POLYMERIZATION
OF THE SAME IN PERFLUOROCARBON LIQUIDS**

Field of the Invention

5 The present invention relates to molecularly
imprinted polymer supports and a method of making these
supports. More specifically the present invention
involves arranging polymerizable functional monomers
around a print molecule using suspension polymerization
10 techniques which is accomplished with a stabilizing co-
polymer having the following formula,



wherein,

X is $\text{C}_n\text{F}_{2n+1}$, $\text{C}_n\text{F}_{2n+1}(\text{CH}_2)_r\text{O}-$, $\text{C}_n\text{F}_{2n+1}\text{O}-$ or
 $\text{C}_n\text{F}_{2n+1}\text{SO}_2\text{N}(\text{C}_2\text{H}_5)\text{C}_2\text{H}_5\text{-O}$;

15 Y is $(\text{Z})_t\text{CH}_3\text{O}(\text{CH}_2\text{CH}_2\text{O})_m\text{C}_2\text{H}_5\text{-O}$ or $(\text{Z})_t\text{CH}_3\text{O}(\text{CH}_2\text{CH}_2\text{O})_m\text{-}$
and Z is a print molecule;

n is between 1 and 20;

m can be zero to about 500;

p is at least 1;

20 q can be zero or any positive number;

r is 1-20, preferably 1 or 2;

s is zero or 1; and

t is zero or 1.

25 The polymer support of the present invention is preferably
in bead form and is capable of separating or resolving
amino-acids, amino-acid derivatives, pharmaceutical
compounds and poly-saccharides.

Background of the Invention

Molecular imprinting, also referred to as templating, has been used for chiral separations and involves arranging polymerizable functional monomers around a print molecule. This is achieved either by utilizing non-covalent interactions such as hydrogen bonds, ion-pair interactions, etc. (non-covalent imprinting), or by reversible covalent inter-actions (covalent imprinting) between the print molecule and the functional monomers. The resulting complexes are then incorporated by polymerization into a highly cross-linked macroporous polymer matrix. Extraction of the print molecule leaves sites in the polymer with specific shape and functional groups complementary to the original print molecule. Mosbach, K., *Trends in Biochemical Sciences*, Vol. 7, pp. 92-96, 1994; Wulff, G., *Trends in Biotechnology*, Vol. 11, pp. 85-87, 1993; and Andersson, et al., *Molecular Interactions in Bioseparations* (Ngo. T.T. ed.), pp. 383-394.

Different racemic compounds have been resolved via molecular imprinting, i.e., "amino-acid derivatives", see Andersson, et al., *Molecular Interactions in Bioseparations* (Ngo T.T. ed.), Plenum Press, pp. 383-394, 1993; "drugs", Fischer, et al., *J. Am. Chem. Soc.*, 113, pp. 9358-9360, 1991; Kempe, et al., *J. Chromatogr.*, Vol. 664, pp. 276-279, 1994; and "sugars", Wulff, et al., *J. Org. Chem.*, Vol. 56, pp. 395-400, 1991; Mayes, et al., *Anal. Biochem.*, Vol. 222, pp. 483-488, 1994. Baseline resolution has been achieved in many cases.

An advantage of molecularly imprinted polymers, in contrast to other chiral stationary phases, is the predictable order of elution of enantiomers. Imprintable supports have been prepared from bulk polymerization techniques, using a porogenic solvent to create a block of macroporous polymer. However, bulk polymerization supports must be crushed, ground and sieved to produce appropriate particle sizes for use in separatory columns and analytical protocols. For example, in chromatographic

evaluations, polymer particles smaller than 25 μm are generally used. However, from the bulk polymerization process the grinding process used to provide these smaller particles from the bulk polymerization process is unsatisfactory. Grinding produces irregularly shaped particles and an excessive and undesirable quantities of "fines." Typically less than 50 percent (50%) of the ground polymer is recovered as useable particles. Irregular particles generally give less efficient column packing for chromatography and often prove troublesome in process scale-up. Hence, uniformly shaped particles, e.g. beaded polymers, would be preferable in most cases. The grinding process also requires an additional treating step to remove the fines, i.e., sedimentation. This is costly and time consuming. The bulk polymerization and necessary grinding process makes this prior art technique labor intensive, wasteful and unacceptable.

Attempts have been made to use suspension and dispersion polymerization techniques for producing beads from acrylic monomers which can contain imprinted molecules. In principle these suspension and dispersion polymerization techniques should offer an alternative to bulk polymerization. However, existing suspension and dispersion techniques are not satisfactory because water or a highly polar organic solvent (e.g. an alcohol) is used as the continuous phase for the relatively hydrophobic monomers. These solvents are incompatible with most covalent and non-covalent imprinting mixtures due to the competition between solvent and functional monomers for specific interaction with the print molecule. Since suspension polymerization techniques use the solvent in large molar excess, the solvents saturate the monomer phase and drastically reduce the number and strength of the interactions between functional monomers and print molecules. In addition, because of the high solubility of acidic monomers in water, random copolymerization of monomers and cross-linker is probably not achieved. Water soluble

print molecules are also lost due to partitioning into the aqueous phase. Not unexpectedly, attempts to make molecularly imprinted polymer beads by suspension polymerization in water have led to only very poor recognition. Damen, et al., *J. Am. Chem. Soc.*, Vol. 102, pp. 3265-3267, 1980; Braun, et al., *Chemiker-Zeitung*, Vol. 108, pp. 255-257, 1984; Byström, et al., *J. Am. Chem. Soc.*, Vol. 115, pp. 2081-2083, 1993. With stable covalent or metal chelate bonds between functional monomers and print molecules prior to polymerization, it may be possible to use aqueous conditions.

Attempts have also been made to produce composite beaded particles by imprinting in the pore network of performed beaded silica, Norrlov, et al., *J. Chromatogr.*, Vol. 299, pp. 29-41, 1984; Wulff, et al., *Reactive Polymers*, Vol. 3, pp. 261-2757, 1985 or TRIM. However, the preparation requires careful handling and the volume of imprinted polymer per unit column is inevitably reduced by the beads themselves.

Sellergren, B., *J. Chromatogr.*, Vol. 673, pp. 133-141, 1994 and Sellergren, B., *Anal. Chem.*, Vol. 66, pp. 1578-1582, 1994, report the use of dispersion polymerization in a polar solvent mixture for molecular imprinting. The process produces random precipitates rather than regular beads. Acceptable results were only achieved for highly charged print molecules, presumably due to the presence of competing solvent effects.

Thus, a need exists for a method that produces beaded polymers containing molecular imprints that is simple and reproducible, does not compromise the quality of the imprints obtained and eliminates the need for grinding and sieving equipment. A need also exists for a molecular imprinted polymer bead that is uniform.

Summary of the Invention

The present invention relates to molecular imprinted polymer supports and their preparation via suspension polymerization. The suspension techniques according to the present invention provide for molecular imprinting by using a perfluorocarbon liquid containing polyoxyethylene ester groups as the dispersing phase. The perfluorocarbon-polyoxyethylene ester containing group compound does not interfere with the interactions between functional monomers and print molecules that are required for the recognition process during molecular imprinting. Controllable "support" particle sizes from about $2\mu\text{m}$ to about $100\mu\text{m}$ are obtained by varying the amount of stabilizing polymer, or agitating technique.

Accordingly, it is an object of the present invention to provide a method that enables imprinted polymers to be easily produced, in beaded form with an almost quantitative yield of useable material.

It is another object of the present invention to provide a fluorocarbon copolymer that stabilizes the emulsion in suspension polymerization processing without interfering with the interactions between functional monomers and print molecules.

It is a still further object to stabilize an emulsion of functional monomers, cross-linkers, print molecules, initiators and porogenic solvents.

Another object of the present invention is to provide molecularly imprinted polymer bead having a size of about 2 to about $100\mu\text{m}$, in high yield.

A still further object of the present invention is to provide small, about $2\text{--}5\mu\text{m}$, beaded packings that provide low back pressure, rapid diffusion, and good separation at high flow rates.

These and other objects and advantages will become more apparent in view of the following description and attached drawings.

Brief Description of the Drawings

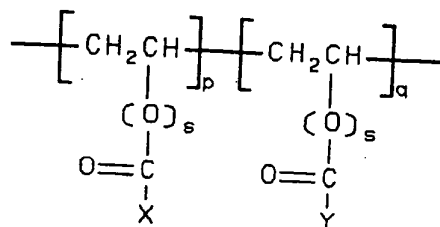
- Figure 1 shows a graph of bead diameter versus PFPS quantity added for a "standard polymerization" containing 1.84g EDMA, 0.16g MAA, 4.2g chloroform and 20mg AIBN emulsified in 20ml PMC.
- Figures 2a-2e show scanning electronmicrographs of beads produced from suspension polymerization in PMC in accordance with the present invention where the beads were placed on aluminum pegs and sputter coated with 15nm gold using a polaron E5150 coater. The images were obtained using an ISI 100A SEM at 25kV. The magnification is 500x. 2(a) PF2; 2(b) PF10; 2(c) PF13; 2(d) PF14; and 2(e) PF15.
- Figure 3 shows an HPLC trace for separation of Boc-D,L-Phe by a 25cm column of Example PF11. Conditions: the column was equilibrated with chloroform (CHCl_3) + 0.1% acetic acid (AA); 20 μg Boc-D,L-Phe was injected and eluted with the same solvent at a flow rate of 0.5mlmin⁻¹. The chromatogram was recorded at 254nm.
- Figures 4a-4e show HPLC traces showing separation of Boc-D,L-Phe by a 25 cm column of Example PF15 (5 μm TRIM beads). Conditions: the column was equilibrated with chloroform + 0.25% acetic acid; 1 mg Boc-D,L-Phe in 20 μl mobile phase was injected and eluted with the same solvent at flow rates of 4(a) 0.5mlmin⁻¹; 4(b) 1mlmin⁻¹; 4(c) 2mlmin⁻¹; 4(d) 3mlmin⁻¹; and 4(e) 5mlmin⁻¹. At 0.5 ml-min⁻¹, $f/g = 0.89$, R_s

= 1.36 and $\alpha = 1.52$ f/g values were
0.89, 0.89, 0.85, 0.76 and 0.61 at
0.5, 1, 2, 3 and 5mlmin⁻¹ respectively.

Detailed Description of the Invention

5 According to the present invention, a suspension
polymerization technique based on emulsion of noncovalent
imprinting mixtures formed in liquid perfluorocarbons that
contain polyoxyethylene ester groups, is provided. Most
prior art suspension and dispersion techniques use water
10 or a highly polar organic solvent (e.g. an alcohol) as the
continuous phase for the relatively hydrophobic monomers,
which is not fully satisfactory. Accordingly, a different
approach is required. The present invention provides the
"different approach." In addition, the drawbacks of prior
15 art processes, e.g., solvents that are incompatible with
most covalent and non-covalent imprinting mixtures due to
the competition between solvent and functional monomers
for specific interaction with the print molecule are
avoided. The present invention avoids the use of
20 dispersants which interfere with the interactions that are
required for recognition between print molecules and
functional monomers. In order to create reasonably stable
emulsion droplets containing monomers, cross-linkers,
print molecules, porogenic solvents, and fluorinated
25 surfactants, the invention uses a perfluorocarbon polymer
that also contains polyoxyethylene ester groups, with or
without a print molecule for providing surface imprinting.

The stabilizing/dispersing agent of the present invention is generally defined by the following formula:



wherein,

X is $\text{C}_n\text{F}_{2n+1}$, $\text{C}_n\text{F}_{2n+1}(\text{CH}_2)_r\text{O}-$, $\text{C}_n\text{F}_{2n+1}\text{O}-$ or $\text{C}_n\text{F}_{2n+1}\text{SO}_2\text{N}(\text{C}_2\text{H}_5)\text{C}_2\text{H}_2-\text{O}$;

Y is $(\text{Z})_t\text{CH}_3\text{O}(\text{CH}_2\text{CH}_2\text{O})_m\text{C}_2\text{H}_2-\text{O}$ or $(\text{Z})_t\text{CH}_3\text{O}(\text{CH}_2\text{CH}_2\text{O})_m-$ and Z is a print molecule;

n is between 1 and 20;

m can be zero to about 500;

p is at least 1;

q can be zero or any positive number;

r is 1-20, preferably 1 or 2;

s is zero or 1; and

t is zero or 1.

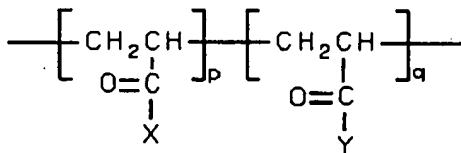
Block, triblock and multiblock copolymers formed from the monomers $\text{C}_n\text{F}_{2n+1}\text{SO}_2\text{N}(\text{C}_2\text{H}_5)\text{C}_2\text{H}_2-\text{OH}$ and $(\text{Z})_t\text{CH}_3\text{O}(\text{CH}_2\text{CH}_2\text{O})_m\text{C}_2\text{H}_2-\text{OH}$ are also within the scope of the present invention. A preferred stabilizing/dispersing agent according to the present invention is a copolymer containing monomer A defined by the formula:

$\text{C}_n\text{F}_{2n+1}\text{SO}_2\text{N}(\text{C}_2\text{H}_5)\text{C}_2\text{H}_2\text{O}-\text{CO}-\text{CH}=\text{CH}_2$ and comonomer B defined by the formula:

$\text{CH}_3\text{O}(\text{CH}_2\text{CH}_2\text{O})_m\text{C}_2\text{H}_2\text{O}-\text{CO}-\text{CH}=\text{CH}_2$;

where n is between 1 and 20 and m is between 0 and 500.

Preferably, n is about 7.5 and m is about 43. Most preferably, the stabilizing/dispersing agent is defined by the following formula,



wherein, X is $\text{C}_n\text{F}_{2n+1}\text{SO}_2\text{N}(\text{C}_2\text{H}_5)\text{C}_2\text{H}_2-\text{O}$;

Y is $\text{ZCH}_3\text{O}(\text{CH}_2\text{CH}_2\text{O})_m\text{C}_2\text{H}_2-\text{O}$ and Z is a print molecule;

n is between 1 and 20;

m can be zero to about 500;

p is at least 1;

q can be zero or any positive number;

s is zero or 1; and

t is zero or 1, and

enables the production of spherical beads in high yields via a suspension polymerization process. Preferably, $m =$ about 7.5, $n =$ about 43, $p =$ about 19 and $q =$ about 1. The values m , n , p , and q need not be whole numbers.

As a result of using the stabilizer of the present invention, the physical characterization of beads is achieved either by utilizing non-covalent interactions such as hydrogen bonds, ion-pair interactions, etc. (non-covalent imprinting), or by reversible covalent interactions (covalent imprinting) between the print molecule and the functional monomers. The complexes formed are then incorporated by polymerization into a highly cross-linked macroporous polymer matrix formed from the copolymerization of different acrylic monomers. Extraction of the print molecule leaves sites in the polymer with specific shape and functional groups complementary to the original print molecule.

The print molecules that can be used in the present invention include, but are not limited to:

1. D- and L-Boc tryptophans
2. D- and L-Boc phenylalanines
3. D- and L-phenylalanines
4. D- and L-Boc-proline-N-hydroxysuccinimide esters

5. D- and L-Cbz tryptophans
6. D- and L-Cbz-aspartic acids
7. D- and L-Cbz-glutamic acids
8. D- and L-Cbz-tryptophan methyl esters
9. nitrophenyl α and β , D-, L-galactosides
10. (S)-(-) timolol
11. D-fructose
12. D-galactose
13. phenyl α -D-mannopyranoside
14. acryl α and β glucosides
15. (R)-phenylsuccinic acid
16. Ac-L-Trp-OEt
17. L-Phe β NA
18. L-LeuAn
19. L-PheAn
20. L-PheGlyAn
21. L-MenPheAn
22. L-PyMePheAn
23. L-PLPheAn
24. N-Ac-L-Phe-L-Trp-OMe
25. diazepam
26. propranolol
27. ephedrine
- However the Boc- D- and L-Phe, and L-Phe have been used in the following non-limiting examples.

REAGENT PREPARATION

MONOMERS

Ethylene glycol dimethacrylate (EDMA) and methacrylic acid (MAA) (Merck, Darmstadt, Germany) were distilled under reduced pressure prior to use. Trimethylolpropane trimethacrylate (TRIM) (Aldrich Chemie, Steinheim, Germany), styrene (Aldrich), methyl methacrylate (MMA) (Aldrich) and benzyl methacrylate (BMA) (Polysciences, Warrington, MA) were used as received. 2,2'-azobis (2-methylpropionitrile (AIBN) came from Janssen Chimica, Goel, Belgium.

2-(N-ethylperfluoroalkylsulphonamido)ethanol (PFA-1) (Fluorochem, Old Glossop, UK), PEG2000 monomethylether (MME) (Fluka Chemic A.G., Buchs, Switzerland) and PEG350MME (Sigma, St. Louis, MO) were converted to their acrylates by reaction with acryloyl chloride and triethylamine in dichloromethane, were also available commercially (from Fluorochem and Polysciences respectively). Perfluoro(methylcyclohexane) (PMC) and Fluorad FC430 were also obtained from Fluorochem.

IMPRINT MOLECULES

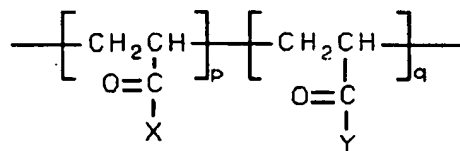
Boc-D-Phe, Boc-L-Phe and Boc-D, L-Phe were obtained from Bachem A.G., Bubendorf, Switzerland). (Boc=tert-butoxy carbonyl; Phe=phenylalanine).

POROGENIC SOLVENTS

Chloroform (CHCl₃) (HPLC grade) was passed down a basic alumina column to remove ethanol and stored over molecular sieves for use as porogenic solvent during imprinting. For HPLC it was used as received. Toluene was dried with sodium and acetonitrile with molecular sieves prior to use. Other solvents were of analytical grade or better and were used as received.

PREPARATION OF STABILIZERS

The most emulsion stabilizing/dispersing polymers (PFPS) according to the present invention are defined by the formula



wherein, X is C_nF_{2n+1}SO₂N(C₂H₅)C₂H₂-O;

Y is Z₁CH₃O(CH₂CH₂O)_mC₂H₂-O and Z is a print molecule;

12

n is between 1 and 20;

m can be zero to about 500;

p is at least 1;

q can be zero or any positive number; and

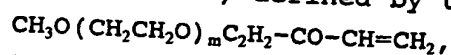
t is zero or 1,

and in particular where n is about 7.5, m is about 43, p is about 19 and q is about 1.

Example 1

4g acryloyl PFA-1 (7.2 mmole)

$C_nF_{2n+1}SO_2N(C_2H_5)C_2H_2-CO-CH=CH_2$ and 0.76g acryloyl PEG2000MME (0.36 mmole) defined by the formula:



were dissolved in 10ml of chloroform, where n is about 7.5 and m is about 43. 24 mg (76 μ mole) of AlBN was added and dissolved oxygen removed by nitrogen sparring for 5 minutes. The tube was then sealed and the contents polymerized at 60°C for 48 hours in a shaking water bath. The resulting solution was slightly turbid and became much more turbid on cooling. Most of the solvent was removed by slow evaporation at 30°C under reduced pressure (to avoid foaming) and the remainder under reduced pressure at 60°C. The resulting polymer was a sticky pale yellow paste which was used without further treatment.

Other polymer stabilizers were synthesized in a similar fashion using the appropriate ratio of monomers and 1 mole % of ATRN. All formed cream to amber polymers varying from glassy to very soft pastes depending on the composition.

Suspension Polymerizations

The amount of porogenic solvent required to just saturate 20 ml PMC was determined. The required amount of PFPs was dissolved in this volume of porogenic solvent in a 50 ml borosilicate glass tube and 20 ml PMC added and shaken to give a uniform white to opalescent emulsion. 5

ml of "imprinting mixture", (Table 1) was added and emulsified by stirring at 2000 rpm for 5 minutes.

TABLE 1

Ex.*	Print Molecule (mg)	MAA (g)	EDMA (g)	Solvent (g)	PFPS (mg)
PF1	Boc-L-Phe (120)	0.16	1.84	CHCl ₃ (4.2)	10
PF2	Boc-L-Phe (120)	0.16	1.84	CHCl ₃ (4.2)	25
PF3	Boc-L-Phe (120)	0.16	1.84	CHCl ₃ (4.2)	50
PF4	Boc-L-Phe (120)	0.16	1.84	CHCl ₃ (4.2)	75
PF5	Boc-L-Phe (120)	0.16	1.84	CHCl ₃ (4.2)	100
PF6	Boc-L-Phe (120)	0.16	1.84	CHCl ₃ (4.2)	200
PF7	Boc-L-Phe (120)	0.16	1.84	CHCl ₃ (4.2)	500
PF8	Boc-L-Phe (120)	0.32	1.84	CHCl ₃ (4.2)	25
PF9	Boc-L-Phe (120)	0.48	1.84	CHCl ₃ (4.2)	25
PF10	Boc-L-Phe (120)	0.64	1.84	CHCl ₃ (4.2)	25
PF11	Boc-L-Phe (68)	0.265	1.84	CHCl ₃ (4.2)	25
PF12	Boc-D,L-Phe (120)	0.16	1.84	CHCl ₃ (4.2)	25
PF13	None	0.16	1.84	toluene (2.45)	25
PF14	None	0.16	1.84	acetone (2.25)	25
PF15	Boc-L-Phe (308)	0.4	1.57 TRIM	CHCl ₃ (4.6)	100
PF16	Boc-L-Phe (308)	0.4	1.57 TRIM	CHCl ₃ (4.6)	25

* The beads prepared according to the present invention, as well as the comparative examples, are designed with the PF code numbers in column 1 of the table.

25 The polymerization apparatus used for all polymerizations in the present invention comprised a 50 ml borosilicate glass tube with screw lid, the center of which was drilled to allow the shaft of a stainless steel flat blade stirrer to pass there through. The stirrer blade

was about one-half the length of the tube. A rubber seal was used to reduce evaporation through this hole. An additional small hole in the lid allowed a nitrogen stream to be fed into the tube via a syringe needle. The tube was held vertically in a retort stand and stirred with an overhead stirrer. A UV lamp was placed about 5 cm away from the tube and the lamp and tube surrounded with aluminum foil to maximize reflected light.

Emulsions designated PF1-PF16 in Table 1 above were prepared and placed in the reactor which was purged with nitrogen for 5 minutes and then the emulsions polymerized by UV irradiation at 366nm at room temperature under a gentle nitrogen stream stirring at 500 rpm. Polymerization was continued for 3 hours. The resulting polymer particles (beads) were filtered on a sintered glass funnel and the PMC recovered. The beads were washed extensively with acetone, sonicating to break up loose aggregates of beads (large aggregates were broken up by gentle crushing with a spatula), before drying and storing.

Varying the amount of PFPS used during the polymerization controls bead size, the relationship being shown in Figure 1. Figure 1 is a graph of the mean and standard deviation for beads made in accordance with the present invention using different amounts of PFPS. A standard was prepared with 2g of monomers in a 5 ml total volume (see Table 1). 10 mg PFPS is at the lower limit of the range where stable emulsions can be formed and quite a lot of aggregate was also present in this sample. Using 150 mg or more of PFPS gave only very small irregular particles of 1-2 μ m. No beads were apparent in these samples.

Comparative Examples

Two commercial fluorinated surfactants (polyfluoroalcohol (PFA-1) and fluorad FC430), a homopolymer of acryloyl PFA-1, a range of random copolymers of acryloyl

PFA-1 with styrene, methyl methacrylate or benzyl methacrylate, and graft copolymers containing perfluoroester groups and PEG groups attached to the main acrylate chain, were also evaluated as stabilizers. Most of these, however, proved to be ineffective. The process was hindered by the high density of the dispersant which caused rapid "creaming" of the emulsion, thus favoring coalescence of dispersed droplets.

Polyacryloyl PFA-1, alone, was evaluated and gave sufficiently stable emulsions for suspension polymerization and gave good quality beads. Unfortunately, due to its poor solubility, this surfactant proved to be extremely difficult to remove from the surface of the beads after polymerization, resulting in extremely hydrophobic surfaces.

The most effective emulsion was achieved using a copolymer of acryloyl-PFA-1 and acryloyl-PEG2000MME (mole ratio 20:1-termed PFPS)

MOLECULARLY IMPRINTED BEAD PROPERTIES

SIZE

The type of emulsification impacts the polymerization process and the resulting molecularly imprinted beads. The preferred method involved stirring at about 2000 rpms for about 5 minutes and gave good uniformity and reproducibility for a bead having a size of between 2 and 25 μ m. Five separate polymerizations performed on different days using 25mg PFPS as the emulsifier gave a mean bead size of 19.7 μ m and a standard error of 0.6 μ m.

Emulsification in an ultrasonic bath for 5 minutes gave a much broader size distribution with an excessive quantity of small particles. Conventional shaking in a tube 3 or 4 times gave good results if larger beads were desired, i.e., 40 μ m to about 100 μ m.

The polymerization temperatures also affected the polymerization process and resulting beads. Attempts to use thermal initiation at 45°C using ABDV as initiator

gave only small irregular fragments. UV initiation of polymerization at 4°C led to a large amount of aggregation. Most polymerizations were performed at ambient temperature (about 20°C), i.e. at least about 18°C, although some temperature increase occurred during polymerization due to the proximity of the UV lamp. Polymerizations carried out during very warm weather, when ambient temperature reached 30°C, gave slightly smaller beads, indicating that polymerization temperature can also impact bead size while still providing reproducible results.

Polymerizations were also carried out in a range of solvents commonly used in molecular imprinting, e.g., chloroform, toluene, acetonitrile and acetone. The polymerization method can use all of these solvents and hence should be appropriate for most imprinting situations. However, the preferred solvents are chloroform, toluene and acetone. The size and surface structure of the beads produced also depends on the porogenic solvent used. Both toluene ($26\mu\text{m} \pm 12\mu\text{m}$ - mean \pm SD) and acetone ($52\mu\text{m} \pm 15\mu\text{m}$) gave larger beads than chloroform ($18\mu\text{m} \pm 8\mu\text{m}$) for 25mg of PFPS in a "standard" polymerization.

Bead Size Distributions

Suspensions of beads in acetone were dried onto microscope slides and about 150 beads measured at random using a calibrated graticule in an optical microscope. Either 100x or 400x magnification was used depending on the particle size. Some samples were also imaged by SEM. Measurements made from these images compared well with the results from optical determinations.

SEM

Polymer beads were placed on aluminum pegs and sputter coated with 15nm gold using a Polaron E5150 gold coater. Images were then obtained using an ISI 100A SEM

at 25kV in order to compare the sizes, surfaces and pore structures of beads produced under different conditions. Scanning electron micrographs of some of the beaded polymer preparations are shown in Figures 2a-2e. The method according to the present invention produces substantially spherical beads, both for EDMA(2a-2d) and TRIM(2e) based polymers, and using a variety of porogenic solvents. The incidence of defects, such as surface indentations or small holes, is somewhat higher than is usually observed for water-based suspension polymerizations in water. The morphology of the beads is typical of beads made by suspension polymerization with a slightly denser and smoother surface layer covering a more porous structure in the interior.

The beads made using acetone as porogenic solvent (Figure 2d) differed from the others. They were larger, had much rougher surface morphology and more "debris" on their surfaces than those prepared using chloroform or toluene. The beads made with toluene as porogenic solvent had a less dense surface shell and somewhat more porous interior structure than those made with chloroform. Figure 2b shows beads of polymer PF9 which has a lower proportion of cross-linker than that in Figure 2a (polymer PF2). The beads in Figure 2b are much more irregular and distorted, suggesting that these particles might remain softer and deformable for longer during polymerization and hence are more prone to distortion due to shear or collision. The internal morphology of these beads also appeared to be more open and porous than that of polymer PF2. This might contribute to the better HPLC performance of the latter. The beads of Fig. 2(c) show PF13 beads and Fig. 2(e) PF15 beads.

HPLC

To confirm that the polymer beads made according to the present invention are molecularly imprinted, and that the quality of the recognition sites is at least as good

as that obtained by traditional bulk polymerization methods, a range of polymers imprinted using Boc-Phe were evaluated by HPLC. This system was chosen since a great deal of information is available on the performance of traditional crushed bulk polymers imprinted with Boc-Phe.

Beads were suspended in a chloroform-acetone (17:3) mixture by sonication and slurry packed into 10 cm by 0.46 cm or 25 cm by 0.46 cm stainless steel columns at 300 bar using an air driven fluid pump and acetone as solvent. The columns were washed with 250 ml methanol:acetic acid (9:1) and then equilibrated with chloroform containing 0.1% or 0.25% acetic acid. 10 μ g Boc D- or L- Phe or 20 μ g racemate in 20 μ l solvent was injected and chromatograms recorded at 254nm at a flow rate of 0.5ml/min. Some separations were also run at higher flow rates and with larger amounts of compound loaded. Chromatographic parameters were calculated using standard theory.

The results for HPLC evaluation of six of the polymers are summarized in Table 2.

TABLE 2

Polymer	Print Molecule/MAA Ratio	% x-link	CHCl ₃ + 0.1% Acetic Acid				
			K'D	K'L	Alpha	Rs	f/g
PF2	1:4	80	0.69	1.44	2.09	0.59	0.51
PF8	1:8	71	0.77	1.42	1.88	0.43	0.37
PF9	1:12	62.5	1.12	1.88	1.68	0.83	0.73
PF10	1:16	56	1.44	2.81	1.81	0.49	0.5
PF11	1:12	75	0.71	1.27	1.8	0.87	0.63
PF11*	1:12	75	0.79	1.43	1.82	1.23	0.84
PF12	1:4	80	0.7	0.7	0	0	0

Polymer	Print Molecule/MAA Ratio	% x-link	CHCl ₃ + 0.25% Acetic Acid				
			K'D	K'L	Alpha	Rs	f/g
PF2	1:4	80	0.43	0.78	1.81	0.26	0.23
PF8	1:8	71	0.54	0.97	1.79	0.31	0.28
PF9	1:12	62.5	0.76	1	1.7	0.69	0.66
PF10	1:16	56	0.5	1.89	1.89	0.48	0.44
PF11	1:12	75	0.63	0.71	1.78	0.48	0.39
PF11*	1:12	75	0.84	1.11	1.91	1.08	0.88
PF12	1:4	80	0	0.54	0	0	0

As the ratio of MAA to print molecule increased the retention times and hence the capacity factors increased due to greater non-specific interaction. The α -values, however, stayed almost constant at about 1.8. This is very similar to values obtained under similar conditions for ground and sieved bulk Boc-Phe polymers (range 1.77 to 2.17). The optimum resolution was found at an MAA:Boc-Phe ratio of 12:1, the value of 0.83 being good for a 10cm column. Using a longer column (25cm) of polymer PF11 resulted in near baseline resolution of the enantiomers as has previously been reported for bulk polymers. The chromatogram for this separation is shown in Figures 3. Polymer PF9, which was only 62.5% cross-linked, performed better than polymer PF11, which had the same print molecule:MAA ratio but was 75% cross-linked. It has previously been shown that separations improve as the degree of cross-linking increases within this range, but this was not observed in these experiments. Polymer PF11 was made using less print molecule (see Table 1) since it is not possible too vary these parameters independently, and it is thus not clear whether the improved resolution was due to the larger number of binding sites or to changes in polymer morphology as a result of the lower cross-linking. Such observations indicate that significant improvements in separation can be achieved by careful optimization of the many compositional and operational variables. The simplicity and speed of the bead polymerization method makes extensive optimization possible.

It has previously been suggested that polymers based on the trifunctional cross-linker TRIM had much better resolution and load capacity than EDMA-based polymers for a range of di and tripeptides. In order to further evaluate the suspension polymerization method according to the present invention, imprints of Boc-L-Phe were made in a TRIM-based polymer. Beads produced using 100mg of PFPS (PF15) had an average diameter of $5.7\mu\text{m}$, and those using 25mg PFPS (PF16) a diameter of $18.8\mu\text{m}$, very similar to

what would have been expected for EDMA-based polymers with the same amount of stabilizing polymer. Thus, in terms of bead-size prediction, TRIM and EDMA seem to behave very similarly. An SEM picture of beads of PF15 is shown in Figure 2e. These beads were tested by HPLC and gave excellent resolution and high load capacities, as was noted for the ground and sieved block polymers. The packed column had very low back pressure, and high resolution could be achieved, even at quite high flow rates.

Figures 4a-4e shows a series of chromatograms for flow rates between 0.5 and 5mlmin⁻¹, i.e., 0.5 ml/min, 1 ml/min, 2 ml/min, 3 ml/min and 5 ml/min. Little difference was observed between 0.5 and 2mlmin⁻¹, suggesting that diffusion rates are rapid for these small beads. The back pressure was very low and resolution excellent ($f/g=0.89$, 0.89 and 0.85 at 0.5, 1 and 2mlmin⁻¹ respectively). Reasonable resolution ($f/g=0.61$) was still achieved at 5mlmin⁻¹ (back pressure 1300psi). Ground and sieved random <25 μ m particles do not usually perform well at flow rates above 1mlmin⁻¹. Working with crushed bulk polymers in the 5 μ m size range is difficult. Extensive defining is required to avoid high back pressures in HPLC columns, and sieves below 10 μ m are unobtainable, making it necessary to use alternative size fractionation techniques. This result indicates that the beaded imprinted polymers should have significant advantages over "traditional" ground and sieved block polymers, both in the case of preparation and in the performance of the resulting columns.

It is also contemplated that because the stabilizer of the present invention is essentially chemically inert, it may be used as a dispersant for making beaded polymers containing water-sensitive monomer units, e.g., acid chlorides or anhydrides.

5 Although the foregoing invention has been described
in some detail by way of illustration and example for
purposes of clarity of understanding, it will be obvious
to those skilled in the art that certain changes and
modifications may be practiced without departing from the
spirit and scope thereof as described in the specification
and as defined in the appended claims.

WE CLAIM:

1. A suspension polymerization stabilizer comprising a copolymer containing monomer units A defined by the formula:

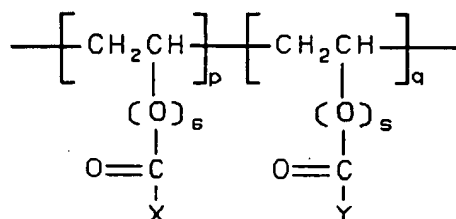
5 $C_nF_{2n+1}SO_2N(C_2H_5)C_2H_2O-CO-CH=CH_2$ and comonomer units B defined by the formula:



where n is between 1 and 20 and m is between 10 and 70 and about 500.

10 2. The stabilizer according to claim 1, wherein n is about 7.5 and m is about 43.

3. A polymer defined by the formula



wherein,

15 X is C_nF_{2n+1} , $C_nF_{2n+1}(CH_2)_rO-$, $C_nF_{2n+1}O-$ or $C_nF_{2n+1}SO_2N(C_2H_5)C_2H_2O-$;

Y is $(Z)_tCH_3O(CH_2CH_2O)_mC_2H_2O-$ or $(Z)_tCH_3O(CH_2CH_2O)_m-$ and Z is a print molecule;

n is between 1 and 20;

m can be zero to about 500;

20 p is at least 1;

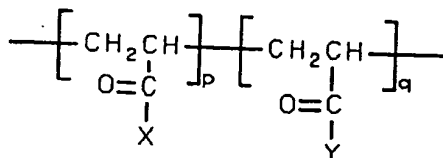
q can be zero or any positive number;

r is 1-20, preferably 1 or 2;

s is zero or 1; and

t is zero or 1.

4. A polymer defined by the formula



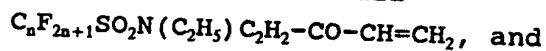
wherein, X is $\text{C}_n\text{F}_{2n+1}\text{SO}_2\text{N}(\text{C}_2\text{H}_5)\text{C}_2\text{H}_2\text{-O}$;
 Y is $\text{CH}_3\text{O}(\text{CH}_2\text{CH}_2\text{O})_m\text{C}_2\text{H}_2\text{-O}$;
 n is between 1 and 20;
 m is between 0 and 500;
 p is at least 1; and q zero or any positive integer.

5. The stabilizer according to claim 1, wherein n is about 7.5 and m is about 43.

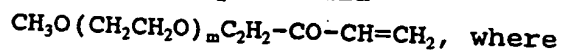
6. A stable emulsion comprising an emulsion of imprinting molecules in liquid perfluorocarbons containing polyoxyethylene ester groups.

7. The emulsion according to claim 6, wherein said liquid fluorocarbon is a copolymer.

8. The emulsion according to claim 7, wherein said copolymer is formed from the polymerization of monomer A and comonomer B, wherein monomer A is defined by the formula



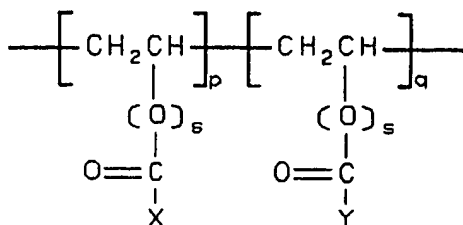
comonomer B is defined by formula



n is between 1 and 20, and m zero to about 500.

25

9. An emulsion comprising imprinting molecules and a polymer defined by the formula



wherein,

X is $\text{C}_n\text{F}_{2n+1}$, $\text{C}_n\text{F}_{2n+1}(\text{CH}_2)_r\text{O}-$, $\text{C}_n\text{F}_{2n+1}\text{O}-$ or $\text{C}_n\text{F}_{2n+1}\text{SO}_2\text{N}(\text{C}_2\text{H}_5)\text{C}_2\text{H}_2\text{O}-$;

Y is $(\text{Z})_t\text{CH}_3\text{O}(\text{CH}_2\text{CH}_2\text{O})_m\text{C}_2\text{H}_2\text{O}-$ or $(\text{Z})_t\text{CH}_3\text{O}(\text{CH}_2\text{CH}_2\text{O})_m-$ and Z is a print molecule;

n is between 1 and 20;

m can be zero to about 500;

p is at least 1;

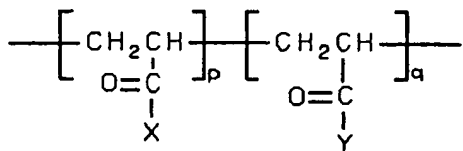
q can be zero or any positive number;

r is 1-20, preferably 1 or 2;

s is zero or 1; and

t is zero or 1.

10. An emulsion comprising imprinting molecules and a polymer defined by the formula



wherein, X is $\text{C}_n\text{F}_{2n+1}\text{SO}_2\text{N}(\text{C}_2\text{H}_5)\text{C}_2\text{H}_2\text{O}-$;

Y is $\text{CH}_3\text{O}(\text{CH}_2\text{CH}_2\text{O})_m\text{C}_2\text{H}_2\text{O}-$;

n is between 1 and 20;

m is zero to about 500;

p is at least 1; and q is zero or any positive

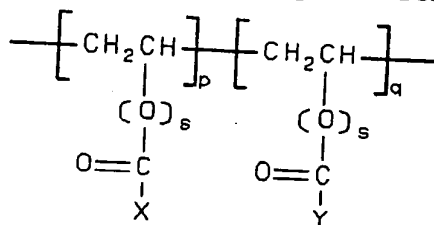
integer.

11. A suspension polymerization process comprising,
- a) forming a reaction mixture containing:
 - 1) a mixture of distinct acrylic monomers;
 - 2) at least one crosslinking agent;
 - 3) at least one initiator;
 - 4) at least one molecular imprint molecule; and
 - 5) at least one porogenic solvent; and,
 - b) polymerizing said reaction mixture in the presence of a stabilizer containing perfluorocarbon and polyoxyethylene ester groups to form molecularly imprinted polymer particles.

12. The process according to claim 11, wherein said stabilizer is chemically inert.

13. The process according to claim 11, wherein said acrylic monomers are methacrylic acid and ethylene glycol dimethacrylate.

14. The process according to claim 11, wherein said stabilizer is a polymer defined by the formula



wherein,

X is $\text{C}_n\text{F}_{2n+1}$, $\text{C}_n\text{F}_{2n+1}(\text{CH}_2)_r\text{O}-$, $\text{C}_n\text{F}_{2n+1}\text{O}-$ or $\text{C}_n\text{F}_{2n+1}\text{SO}_2\text{N}(\text{C}_2\text{H}_5)_2\text{C}_2\text{H}_2\text{O}-$;

Y is $(\text{Z})_l\text{CH}_3\text{O}(\text{CH}_2\text{CH}_2\text{O})_m\text{C}_2\text{H}_2\text{O}-$ or $(\text{Z})_l\text{CH}_3\text{O}(\text{CH}_2\text{CH}_2\text{O})_m-$ and Z is a print molecule;

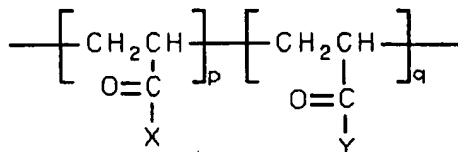
n is between 1 and 20;

m can be zero to about 500;

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p is at least 1;
 q can be zero or any positive number;
 r is 1-20, preferably 1 or 2;
 s is zero or 1; and
 t is zero or 1.

15. The process according to claim 11, wherein said stabilizer is a polymer defined by the formula



wherein, X is $\text{C}_n\text{F}_{2n+1}\text{SO}_2\text{N}(\text{C}_2\text{H}_5)\text{C}_2\text{H}_2\text{-O}$;
 Y is $\text{CH}_3\text{O}(\text{CH}_2\text{CH}_2\text{O})_m\text{C}_2\text{H}_2\text{-O}$;
 n is between 1 and 20;
 m is zero to about 500;
 p is at least 1; and
 q is zero or any positive number.

16. A process according to claim 11, wherein prior to polymerization an emulsion is formed from components 1-5 and said stabilizers.

17. A process according to claim 16, wherein the reaction mixture is mixed for approximately 5 minutes at about 2000 rpms to form said emulsion.

18. A process according to claim 16, wherein the polymerization is conducted at temperatures between less than about 20°C and about 30°C.

19. A process according to claim 11, wherein said polymerization is initiated by ultraviolet radiation.

20. A process according to claims 11, 14 or 15, wherein said polymer particles are beads.

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21. A process according to claim 12, wherein said polymer particles provide a uniform surface for reproducible surface presentation.

5 22. A process according to claim 17, wherein said beads are spherical in shape.

23. A process according to claim 20, wherein said beads have a diameter of from about 2 to 100 microns.

24. A process according to claim 20, wherein said beads have a diameter of between 2 and 50 microns.

10 25. A process according to claim 20, wherein said beads have a diameter of between about 5 and 25 microns.

26. A process according to claim 11, wherein said porogenic solvent is selected from the group consisting of acetone, chloroform and toluene.

15 27. A process according to claims 11, 14 or 15, wherein said molecular imprint molecule is capable of separating or resolving amino-acids, amino-acid derivatives, pharmaceutical compounds and polysaccharides.

20 28. A process according to claims 11, 14 or 15, wherein said molecular imprint molecule is selected from D- and L-Boc amino acids and their derivatives.

25 29. A molecularly imprinted support formed from at least two distinct acrylic monomers and at least one imprinted molecule to form beads having a uniform surface for reproducible surface presentation.

30. A particle according to claim 29, wherein said beads are spherical in shape.

31. A particle according to claim 30, wherein said beads have a diameter of from about 2 to 100 microns.

32. A particle according to claim 31, wherein said beads have a diameter of between 2 and 50 microns.

5 33. A particle according to claim 32, wherein said beads have a diameter of between about 5 and 25 microns.

34. A particle according to claim 29, wherein said acrylic monomers are methacrylic acid and ethylene glycol dimethacrylate.

10 35. A particle according to claim 29, wherein said molecular imprint molecule is capable of separating or resolving amino-acids and amino-acid derivatives, pharmaceutical compounds and polysaccharides.

15 36. A particle according to claim 35, wherein said molecular imprint molecule is selected from D- and L-Boc-amino acids and their derivatives.

37. A process according to claims 14 or 15, where s is zero.

38. A process according to claim 14, wherein s is 1.

20 39. A process according to claim 14, where t is 1.

25 40. A process according to claim 14, wherein said print molecule is selected from D- and L-Boc tryptophans, D- and L-Boc phenylalanines, D- and L-phenylalanines, D- and L-Boc-proline-N-hydroxysuccinimide esters, D- and L-Cbz tryptophans, D- and L-Cbz-aspartic acids, D- and L-Cbz-glutamic acids, D- and L-Cbz-tryptophan methyl esters, nitrophenyl α and β , D-, L-galactosides, (S)-(-) timolol,

5 D-fructose, D-galactose, phenyl α -D-mannopyranoside, acryl
 α and β glucosides, (R)-phenylsuccinic acid, Ac-L-Trp-OEt,
L-Phe β NA, L-LeuAn, L-PheAn, L-PheGlyAn, L-MenPheAn, L-
PyMePheAn, L-PLPheAn, N-Ac-L-Phe-L-Trp-OMe, diazepam,
propranolol and ephedrine.

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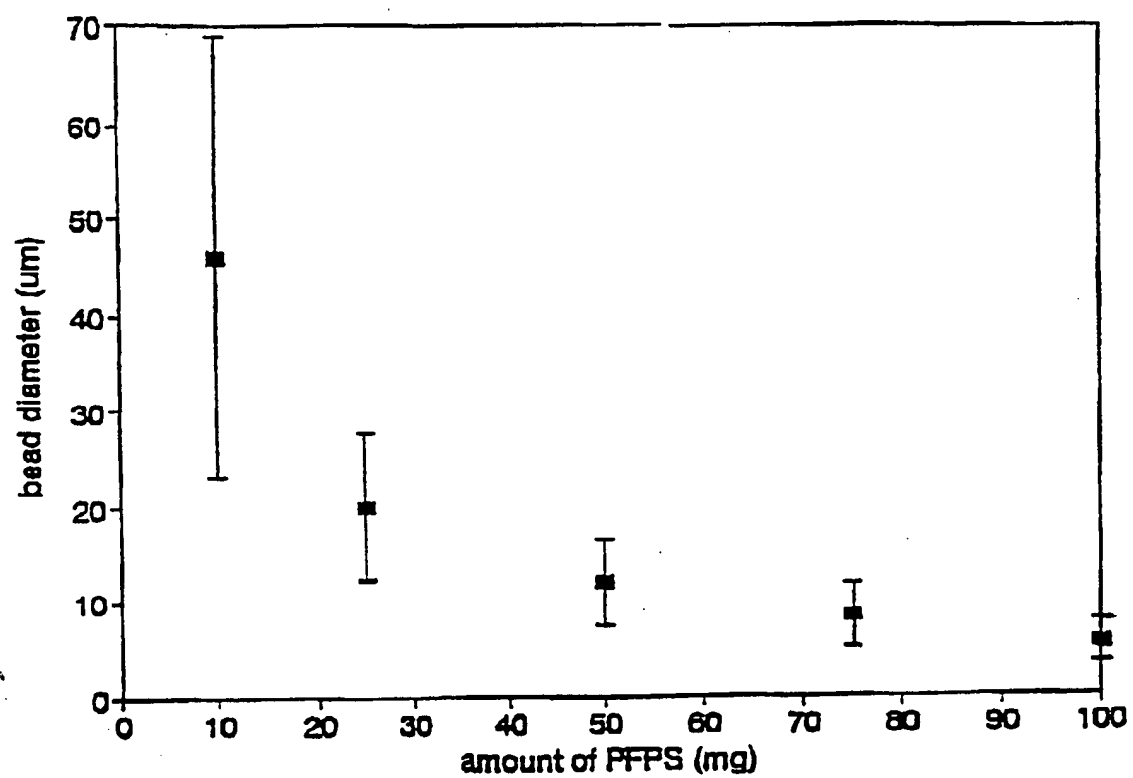


FIGURE 1

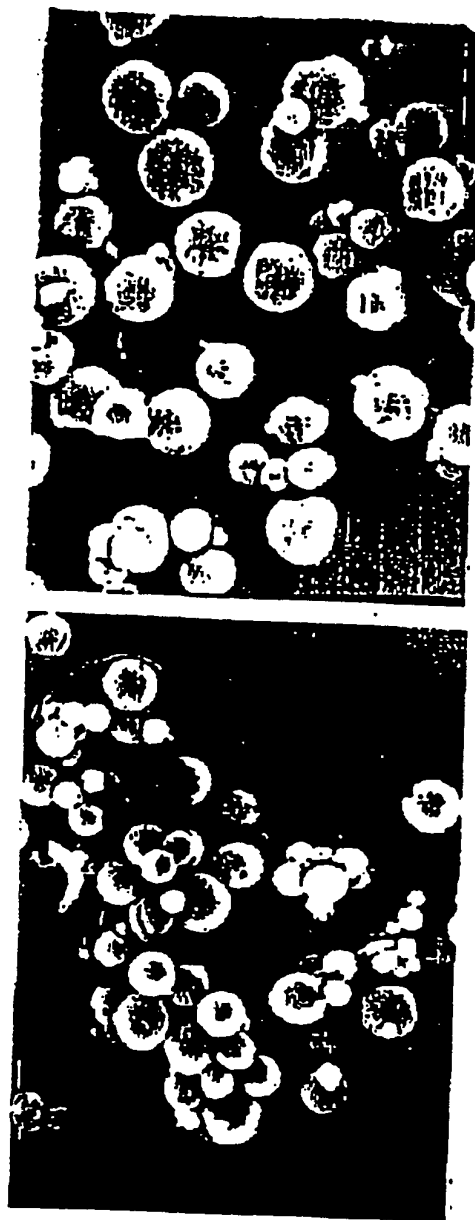


FIGURE 2(a)

FIGURE 2(b)



FIGURE 2(d)

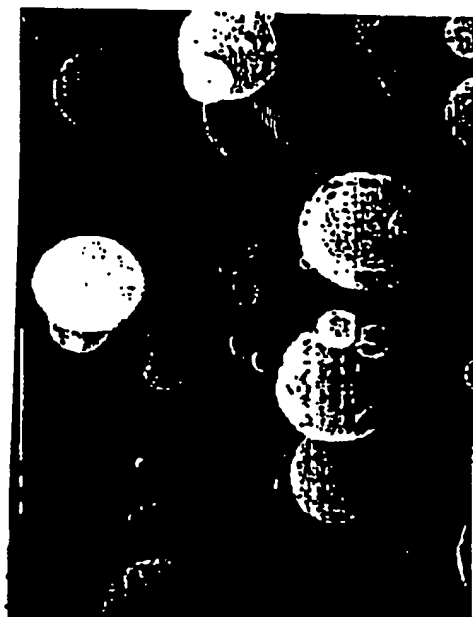


FIGURE 2(c)

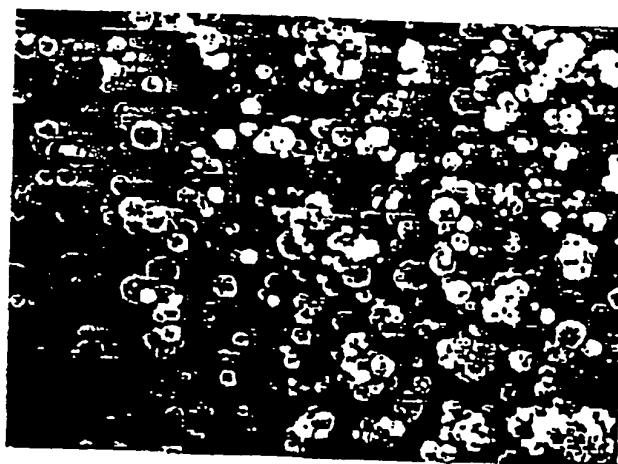


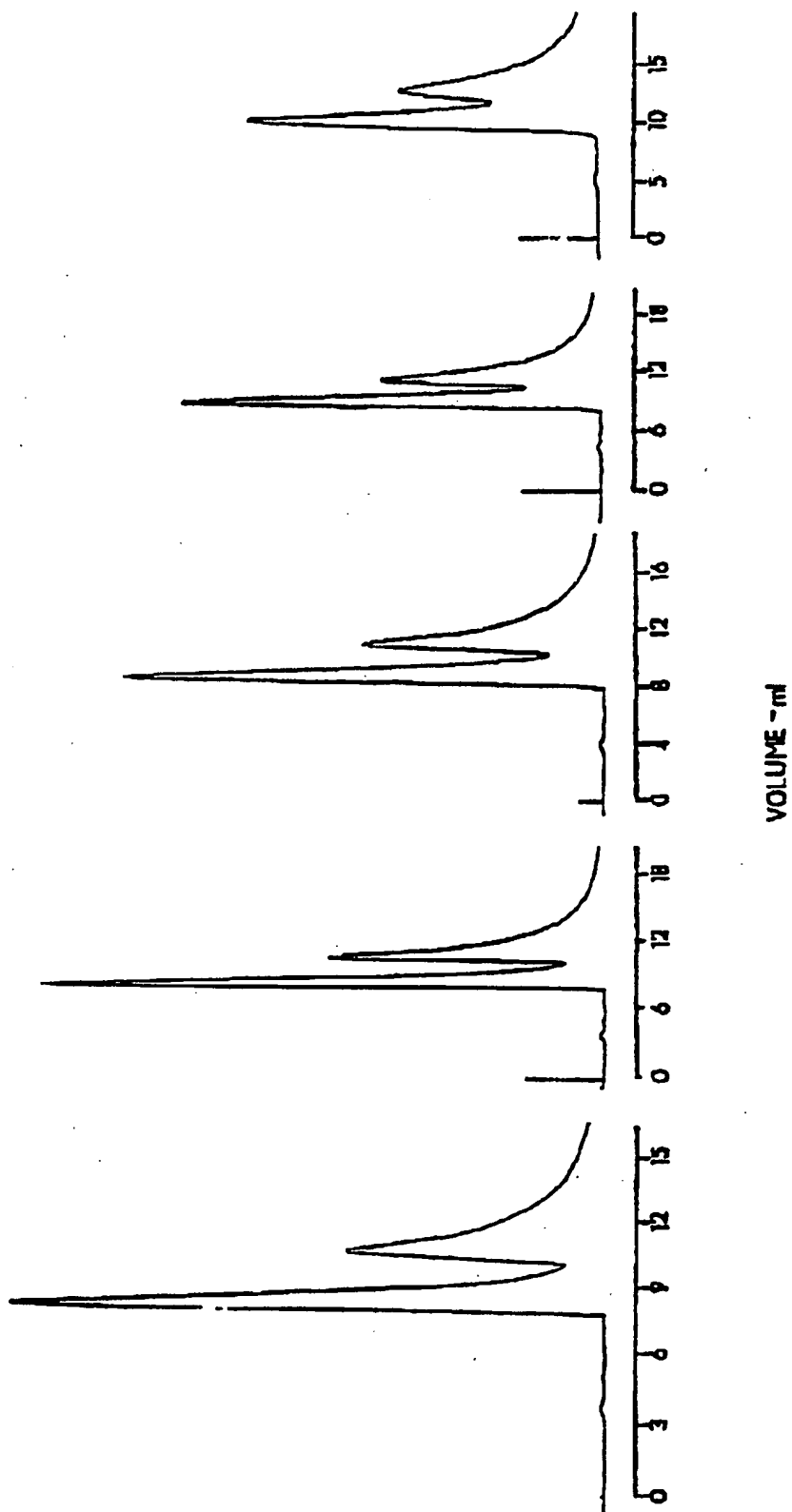
FIGURE 2(e)



FIGURE 3

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FIGURE 4(a) FIGURE 4(b) FIGURE 4(c) FIGURE 4(d) FIGURE 4(e)



INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 96/06972

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C08F220/38 C08F220/28 C07B57/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C08F C07B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US,A,3 654 244 (A.G. PITTMAN) 4 April 1972 see claims 1,4	1
A	DE,A,23 58 647 (ASAHI GLASS CO. LTD.) 30 May 1974 see page 3, line 11 - page 3, line 30; claim 1	1
A	WO,A,90 07527 (KODAK LTD.) 12 July 1990	
A	WO,A,94 14835 (MOSBACH K.) 7 July 1994	

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☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

13 September 1996

Date of mailing of the international search report

- 3. 10. 96

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European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl,
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INTERNATIONAL SEARCH REPORT

Information on patent family members

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PCT/US 96/06972

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		JP-A- 49075472	22-07-74
		JP-B- 52035033	07-09-77
		FR-A- 2255321	18-07-75
		GB-A- 1431974	14-04-76
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		JP-T- 8507291	06-08-96

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